## PEER REVIEW HISTORY



## ARTICLE DETAILS

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| **TITLE (PROVISIONAL)** | Prevalence of hepatitis C virus infection and its correlates in a rural area of southwestern China: a community-based cross sectional study |
| **AUTHORS** | Cheng, Wanting; Yang, Ya; Zhou, Yibiao; Xiao, Penglei; Shi, Yan; Gao, Jianchuan; Chen, Yue; Liang, Song; Yihuo, Wu-Li; Song, Xiuxia; Jiang, Qingwu |

## VERSION 1 - REVIEW

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| **REVIEWER** | Seng Gee Lim<br>National University Health System<br>Singapore |
| **REVIEW RETURNED** | 01-Feb-2017 |

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| **GENERAL COMMENTS** | This was a cross sectional study of HCV prevalence in Yi county in China, an underdeveloped region with high illiteracy rate, high drug abuse and HIV infection and were predominantly farmers. The authors found that the overall HCV prevalence was 2.8% considerably higher than in China overall with risk factors being male, married, history of blood transfusion or drug abuse as risk factors. Similar findings were seen in those with HIV infection.<br><br>Comments<br>1. The study design was commendable for a community based study with random selection of villages for testing reducing the risk of bias in this rather difficult to perform study<br>2. However there is no flow chart on how many patients refused testing, how many residents of each village were untested for various reasons so we can understand the completeness of the survey<br>3. HCV RNA testing was performed but this was not presented. Was the HCV positive patients all HCV RNA positive? The false positive rate of the anti-HCV test should also be stated since the ELISA test was not a recognized test outside China<br>4. The main question is how the authors plan to use the study findings. Would this study enable health authorities to focus on a risk factor screening strategy? |

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| **REVIEWER** | Niklas Luhmann<br>Médecins du Monde/Doctors of the World Paris<br>France |
| **REVIEW RETURNED** | 14-Feb-2017 |

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| **GENERAL COMMENTS** | this is an interesting paper but I find it needs quite a major revision. in the current form I find it not particularly concvincing |

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| | here below some of my comments and observations:<br><br>Major:<br><br>- the methodology section should develop in more detail how counties and towns were selected and out of how many overall. It is not entirely clear if the sample was representative of the entire region.<br><br>- in addition, it is not clear enough if you have a rationale around assessing HCV in the Yi minority or if they just happen to live in this region- and how this relates or not to the sample methodology<br><br>- it is as well not clear enough how sampling within the towns proceeded; you refer to the the support of village chiefs and I get the impression sampling was supposed to be exhaustive? in any case there maybe an important bias around sample methdodology, but its not easy to judge.<br><br>- I think the cut off of 14 years in your results section needs a bit more explanation; why was it chosen; why is that criteria so important and why is it different of your selection criteria etc. (especially as all individuals/participants under 18 needed parental consent and that you included al individuals that were aged over 6 years)<br><br>- in general the entire manuscript including the abstract needs review for language; often the use of past tense is not correct and there are other major errors<br><br>- i would like to see some more details about the quality and the performance characteristics of the RDT that was used for antibody detection<br><br>- it seems not clear why you have not included the results for RNA testing - for all participants with positive antibodies; this would make the papaer more interesting as we finally want to know the epidemic of chronic hepatitis<br><br>- as you state yourself a cross sectional survey does not allow to really conclude on casual relationships, i would recommend to use "associated factors" throughout the entire manuscript<br><br>- in the discussion do you compare the prevalence to the one found in rest of china in the same group (14 years or older)? please clarify what exactly you compare to and how this is interpreted<br><br>- it would make sense as well to discuss if other regions in China that report a lot of drug use have similar prevalence rates<br><br>- I dont really find it convincing that the difference between male and female prevalence is biological; in my eyes it is more likely that it is due to drug use history<br><br>- the paragraph on MSM is not particularly convincing<br><br>- very importantly in discussion and conclusion theres not enough reference made to evidence based interventions for people who use |

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| | and inject drugs; banning drug trade and drug use is not a sufficiently evidence based intervention and the authors should refer to needle and syringe programs, methadone/opiate substitution therapy and treatment access of drug users<br><br>- i find as well discussion and conclusion are a lot reflecting issues regarding the associated factors with HCV infection; this study is not developed to allow confirmation of HCV transmission routes but rather an epidemiological study; thus epidemiological findings should be discussed more in detail<br><br>Minor:<br>- introduction line 21: with new DAAs treatment outcomes for HCV moninfection and HIV/HCV infection are very similar<br>- some references - especially in the introduction - could be more up to date |

**VERSION 1 – AUTHOR RESPONSE**

Reviewer #1:
This was a cross sectional study of HCV prevalence in Yi county in China, an underdeveloped region with high illiteracy rate, high drug abuse and HIV infection and were predominantly farmers. The authors found that the overall HCV prevalence was 2.8% considerably higher than in China overall with risk factors being male, married, history of blood transfusion or drug abuse as risk factors. Similar findings were seen in those with HIV infection.
We are really grateful for your positive evaluation. According to your comments, we have amended it and expressed it clearly and strictly. See the following point-by-point responses.

Comments
1. The study design was commendable for a community based study with random selection of villages for testing reducing the risk of bias in this rather difficult to perform study
Response: Thank you very much for your encouraging and positive comments. We do hope our study could help to understand the epidemiology of HCV infection and to come up with control measures to lower the burden of HCV infection in this region.

2. However there is no flow chart on how many patients refused testing, how many residents of each village were untested for various reasons so we can understand the completeness of the survey
Response: A flow chart has been added into the Method section.（Page 4, line 10）

3. HCV RNA testing was performed but this was not presented. Was the HCV positive patients all HCV RNA positive? The false positive rate of the anti-HCV test should also be stated since the ELISA test was not a recognized test outside China
Response: Thank you for your critical suggestion. We agreed that HCV RNA is needed to confirm the HCV prevalence. Unfortunately only 69.01 %( 46/71) agreed to provide a 5ml blood sample for HCV RNA testing. These data indicated 71.7 %( 33/46) had active HCV infection which has been added in the revised manuscript. （Page , lines ）Although we don't know the false positive rate of anti-HCV according to HCV RNA test, a study found that the HCV colloidal gold kits have a low false positive rate. （Page 6, lines 9-12; Page 7, lines5-10）
(Yao RN, et al. Comparison of HCV Colloidal Gold Reagent Fast Examination and Conventional Method in Emergency Surgery on Patients before Transfusion. Journal of Clinical Transfusion & Laboratory Medicine, 2009.10(4), 289-291)

4. The main question is how the authors plan to use the study findings. Would this study enable health authorities to focus on a risk factor screening strategy?
Response: Thanks for your thoughtful suggestion. Our study confirmed a high prevalence of HCV infection and identified its correlates in the Yi population in this region, which indicated screening and health education among persons at risk, especially those with drug abuse, are necessary in order to prevent further HCV transmission. Chinese health government recommends identification and testing of persons at risk as well as surveillance and research to monitor disease trends. Health authorities are developing a risk factor screening strategy. Further research is needed to evaluate the cost and benefit of HCV screening strategy based on persons at risk. Changes have been made in our revised manuscript. (Page 7, lines 27-29)
(National Health and Family Planning Commission of the People's Republic of China. Screening and management of viral hepatitis C [J]. Infectious disease information, 2015(1):1-2).

Reviewer #2:
This is an interesting paper but I find it needs quite a major revision. In the current form I find it not particularly convincing

Here below some of my comments and observations:

Major:

- The methodology section should develop in more detail how counties and towns were selected and out of how many overall. It is not entirely clear if the sample was representative of the entire region.
Response: Thank you for your comments. Four towns from 3 counties were included in our study. The study adopted a two-stage sampling scheme. In the first stage, 3 counties (P, Z, and M) were selected from the Yi Prefecture on the basis of similar social demographic characters (such as gender structure, economic status, custom, education level and sanitary facility) as well as high prevalence of HIV infection. In the second stage, 4 towns were randomly selected from 3 counties (A and B from P County, C from Z county and D from M county) and all 27 villages of these towns were investigated. Accordingly, we have provided more detail information on study design and a flow chart in our revised manuscript. （Page 4 , lines 5-10 ）

- in addition, it is not clear enough if you have a rationale around assessing HCV in the Yi minority or if they just happen to live in this region- and how this relates or not to the sample methodology
Response: Thank you for your thoughtful and professional suggestion. This region is noted for high risks of drug abuse and HIV infection. Our aim was to investigate the prevalence and correlates of HCV infection in this area. All local villagers were invited to participate if they aged ≥ 6 years and lived there for more than 6 moths in the year prior to the survey. Considering the geographic characteristic of this area (rugged mountainous terrain and sparsely scattered population) and high authority of village chiefs, village chiefs were first contacted and were responsible for gathering eligible villagers to township hospital when they came for our survey. The information has been added to the revision. （Page 4, lines11-14）

- it is as well not clear enough how sampling within the towns proceeded; you refer to the support of village chiefs and I get the impression sampling was supposed to be exhaustive? In any case there may be an important bias around sample methodology, but it's not easy to judge.
Response: Thank you very much for your suggestion. We have provided more detail information about the study design and a flow chart to make a clarification. Despite the support and cooperation of village chiefs, some residents had drifted to the cities for working opportunities and therefore missed our investigation. We have described this limitation in the Discussion section. （Page 9, lines 6-8）

- I think the cut off of 14 years in your results section needs a bit more explanation; why was it chosen; why is that criteria so important and why is it different of your selection criteria etc. (especially as all individuals/participants under 18 needed parental consent and that you included al individuals that were aged over 6 years)
Response: Thank you for your valuable questions. Yi indigenous culture considers 14 years of age as the start of adulthood for permitting sexual behaviors, and we found the proportion of anti-HCV was very low under age 14. Therefore, we include participants of 14 years or older into our analysis despite that we provided questionnaire interviewing and anti-HCV testing for all participants. Please find the changes in the revised manuscript.（Page 6, lines 2-5）

- in general the entire manuscript including the abstract needs review for language; often the use of past tense is not correct and there are other major errors ：
Response: Thank you for your careful reviewing our manuscript and pointing out this important issue. Large efforts have been made to improve the language. We have read the manuscript carefully and corrected some misspellings and grammar errors. Please seen in the revised manuscript.

- i would like to see some more details about the quality and the performance characteristics of the RDT that was used for antibody detection
Response: Thanks for your thoughtful suggestion. HCV antibody was tested by Diagnostic Kit for Hepatitis C Virus Antibody (Colloidal Gold). Product specifications showed that the sensitivity and specificity of Colloidal Gold Kit are both higher than 95%. The conformity rate of colloidal gold paper method and HCV RNA test was found to be 100. 0% (Yao R N, et al. Comparison of HCV Colloid Gold Reagent Fast Examination Method and Conventional Examination Method in Blood Donors [J]. Journal of Clinical Transfusion & Laboratory Medicine, 2010, 12(4), 300-301,305). （Page 4, lines 28-30）

- it seems not clear why you have not included the results for RNA testing - for all participants with positive antibodies; this would make the paper more interesting as we finally want to know the epidemic of chronic hepatitis
Response: Thanks for your good questions. We agreed that HCV RNA is needed to confirm the HCV prevalence. However, only 46 out of 71 agreed to provide 5ml blood samples for HCV RNA testing. These data indicated that 71.7 %( 33/46) had active HCV infection. We have added these results in the revised manuscript. （Page 6, lines 9-12; Page 7, lines5-10）

- as you state yourself a cross sectional survey does not allow to really conclude on casual relationships, i would recommend to use "associated factors" throughout the entire manuscript
Response: Thank you for your thoughtful suggestion. Changes have been made as suggested.

- in the discussion do you compare the prevalence to the one found in rest of china in the same group (14 years or older)? Please clarify what exactly you compare to and how this is interpreted
Response: Thank you for your suggestion. We were not able to make such a comparison due to a lack of data. Our prevalence estimated in this area was considerably higher than the national level (all age group: 0.58%), which implied that drug abuse is a major correlate of HCV. Please find these additions in the Discussion section. （Page 6, line 29; page 7, lines 1-2）

- it would make sense as well to discuss if other regions in China that report a lot of drug use have similar prevalence rates.
Response: Thanks for your valuable suggestion. Zhuo et al studied the prevalence of HCV infection in a rural area with high drug abuse in Dehong prefecture, Yunnan province, China and found that the prevalence of HCV infection was 4.3%。Most previous studies focused on high risk groups, few studies were designed to investigate the overall prevalence and epidemiology of HCV of the general population in an area with high drug abuse and HIV infection. The information has been added it in

the Discussion section. （Page 7, lines 4-5）
(Zhuo L R, et al. Prevalence and correlation of hepatitis C virus infection among ethnic minority villagers in Dehong prefecture, Yunnan province [J]. Chinese Preventive Medicine, 2012, 24(24):1491-500.)

- I don't really find it convincing that the difference between male and female prevalence is biological; in my eyes it is more likely that it is due to drug use history
Response: We agree. We have added that the sex difference in the prevalence is likely due to drug abuse. （Page 8, lines 3-4）

- the paragraph on MSM is not particularly convincing
Response: Thank you for your careful reviewing our manuscript and pointing out this issue. 'MSM is rare in this area' was told by village chiefs rather than verified documents. Accordingly, we removed this section.

- very importantly in discussion and conclusion there is not enough reference made to evidence based interventions for people who use and inject drugs; banning drug trade and drug use is not a sufficiently evidence based intervention and the authors should refer to needle and syringe programs, methadone/opiate substitution therapy and treatment access of drug users
Response: Thank you for your suggestions, and we have modified the Discussion section accordingly. （Page 7, lines 19-29）

- i find as well discussion and conclusion are a lot reflecting issues regarding the associated factors with HCV infection; this study is not developed to allow confirmation of HCV transmission routes but rather an epidemiological study; thus epidemiological findings should be discussed more in detail
Response: Thank you for your critical suggestion. Changes have been made for the Discussion and Conclusion sections.

Minor:
- introduction line 21: with new DAAs treatment outcomes for HCV moninfection and HIV/HCV infection are very similar
Response: Thank you very much for your correction. We have read the 'Guidelines for the screening, care and treatment of persons with chronic hepatitis C infection: updated version' carefully, and made necessary changes.

- some references - especially in the introduction - could be more up to date
Response: New references have been added.

**VERSION 2 – REVIEW**

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| **REVIEWER** | Seng Gee Lim<br>National University Health System<br>Singapore |
| **REVIEW RETURNED** | 14-Apr-2017 |

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| **GENERAL COMMENTS** | No additional changes required |

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| **REVIEWER** | Niklas Luhmann<br>Médecins du Monde<br>France |
| **REVIEW RETURNED** | 28-Apr-2017 |

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| **GENERAL COMMENTS** | thank you for your great efforts to review this paper and to integrate my suggestions. the paper is now of higher quality and I have only some rather minor remaining suggestions to share. please integrate these and I will not need to review again<br><br>- throughout all parts please specify when using "HCV infection" that you refer mainly to HCV antibody prevalence or HCV exposure (including in the abstract line 16 etc.)<br>- throughout the paper it would be Worth to specify more clearly what exactly you refer to when you do refer to "drug abuse" and "blood transfusion: is it "ever" used injection drugs" or "currently/recently injecting"? is it "ever having undergone a blood transfusion?"<br>- in the abstract conclusion refer first to the results of finding a rather high prevalence - when compared to rest of China (line 27 abstract)<br>- source 2 and 3 are too old. Please refer to the latest WHO World hepatitis report for more data and latest references<br>- ideally i would suggest that the final version is read by someone with mother tongue English in order to just to make it completely clean on a linguistic level<br>- results line 14 and 15; it is interesting to refer to the knowledge of HCv status and treatment access; but I dont understand the way you refer to as "data not shown"; seems potentially confusing to me<br>- discussion line line -/ not sure what the frequency of drug abuse exactly refers to ? see comment higher up<br>-overall the weakest part of the paper is now the discussion: a;) i would not refer to the HCV PCR positivity in the discussion, your point here is confusing; b.) if you have data from the China sero-prevalence Survey for participants older than 14 (reference 19) than compare to this group. And make sure this is as well antibody positivity rate; c.) i would strongly suggest to integrate a bit more about HCV treatment for people who inject/use drugs as a strategy to have an impact on the epidemic and thus refer to the import(ance to integrate PWID in treatment and screening efforts; d.) I guess the part on blood transfusion and married status could be still a bit improved; e.) could you still improve the discussion around the HIV/HCV co-infections results please; its a bit unspecific and confusing<br>- refer to scale up of prevention and treatment in conclusion |

## VERSION 2 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Seng Gee Lim
Institution and Country: National University Health System, Singapore
Competing Interests: none

No additional changes required
Response: We thank Prof. Lim for his valuable suggestions and comments.

Reviewer: 2
Reviewer Name: Niklas Luhmann
Institution and Country: Médecins du Monde, France
Competing Interests: None declared

Thank you for your great efforts to review this paper and to integrate my suggestions. the paper is now of higher quality and I have only some rather minor remaining suggestions to share. please

integrate these and I will not need to review again

- throughout all parts please specify when using "HCV infection" that you refer mainly to HCV antibody prevalence or HCV exposure (including in the abstract line 16 etc.)
Response: Thank you for your comments. We have amended it according to your suggestion.

- throughout the paper it would be Worth to specify more clearly what exactly you refer to when you do refer to "drug abuse" and "blood transfusion: is it "ever" used injection drugs" or "currently/recently injecting"? is it "ever having undergone a blood transfusion?"
Response: Thank you very much for your suggestion. We changed “drug abuse” to “ever using injection drug”, and “blood transfusion” to “ever having blood transfusion” in revised manuscript.

- in the abstract conclusion refer first to the results of finding a rather high prevalence when compared to rest of China (line 27 abstract)
Response: Thank you very much for your suggestion. We have amended it in our revised manuscript. （Page 2, line 29 ）

- source 2 and 3 are too old. Please refer to the latest WHO World hepatitis report for more data and latest references
Response: Changes have been made as suggested. （Page 3, line 10-12）

- ideally i would suggest that the final version is read by someone with mother tongue English in order to just to make it completely clean on a linguistic level.
Response: Thank you for your careful reviewing our manuscript and pointing out this important issue. We have read our manuscript thoroughly and made some changes. The new manuscript has been proofed and polished by Prof. Yue Chen.

- results line 14 and 15; it is interesting to refer to the knowledge of HCv status and treatment access; but I dont understand the way you refer to as "data not shown"; seems potentially confusing to me
Response: Thank you very much for your suggestion. Such information was not gathered by questionnaire investigation but by chatting with those HCV-infected individuals. Therefore, we cannot give the specific proportions.

- discussion line line -/ not sure what the frequency of drug abuse exactly refers to ? see comment higher up
Response: Drug abuse refers to “ever using injection drug”. We have amended it accordingly.

- overall the weakest part of the paper is now the discussion: a;) i would not refer to the HCV PCR positivity in the discussion, your point here is confusing; b.) if you have data from the China sero-prevalence Survey for participants older than 14 (reference 19) than compare to this group. And make sure this is as well antibody positivity rate (whole population; antibody positivity) ; c.) i would strongly suggest to integrate a bit more about HCV treatment for people who inject/use drugs as a strategy to have an impact on the epidemic and thus refer to the importance to integrate PWID in treatment and screening efforts; d.) I guess the part on blood transfusion and married status could be still a bit improved; e.) could you still improve the discussion around the HIV/HCV co-infections results please; its a bit unspecific and confusing
Response: Thank you for your thoughtful suggestion. Changes have been made as suggested. a) We removed this section; b) we supplemented this data about prevalence of HCV antibody aged 15~59 years in China; c) we added it accordingly. (Page 8, line 3-4); d) and e) we read and revised this part carefully. Seen in the Discussion section.

- refer to scale up of prevention and treatment in conclusion

Response: Thanks very much for your good suggestion. We have amended it according to your suggestion. （Page 9, line 18-20）